JOURNAL OF FORENSIC SCIENCES

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Journal:	*Journal of Forensic Sciences*
Manuscript ID	JOFS-20-637.R1
Manuscript Type:	Technical Note
Keywords:	Forensic anthropology, Cut marks, Bone, Kerf, Mechanical properties, Porcine

Validity of the use of porcine bone in forensic cut mark studies*,†

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*Funded by the University of Lincoln.

†Use of animal subjects: All procedures were performed in compliance with relevant laws and institutional guidelines, and were approved by the University of Lincoln Research Ethics Committee.

Running head: Porcine bone in cut mark studies

Abstract

Porcine bone is often used as a substitute for human bone in forensic trauma studies, but little has been published on its comparative mechanical behavior. The factors affecting mechanical properties and therefore selection of bone models are complex and include the age of the animal at death, and physiological loading conditions, the latter being of particular relevance when using a quadrupedal animal as a human substitute. The regional variation in hardness of adult and infant porcine bones was investigated using Vickers' indentation tests and compared to published data for human limb bones to relate differences to inherent genetic effects and loading influences, and to examine the validity of the porcine-human model. Significant differences in hardness were observed both along and around the adult porcine humerus and femur, but no significant differences were found along the length of the infant bones. Significant differences were found between the fore-and hind-limb, but only in the infant specimens. The hardness values for porcine adult cortical bone from the femur (52.23 ± 1.00 kg mm^{-2}) were comparable to those reported in the literature for adult human cortical bone from the fibula, ilium and calcaneus. These data will help inform subject selection in terms of both species and bone type for use in future trauma studies.

KEYWORDS

forensic anthropology, cut marks, bone, porcine, kerf, hardness, mechanical properties

Highlights

- Infant pigs showed different hardness in fore and hind limbs, adult pigs did not.
- Adult pigs showed differences in hardness around and along the length of the humerus and femur.
- Infant pigs did not show significant variation in hardness along the fore or hind limb.
- Effects of age and bone type should be considered when selecting samples for cut mark studies.
- Porcine bone has similar hardness to human bone making it a useful substitute in cut mark studies.

Introduction

Due to the ethical, legal and practical use of Post Mortem Human Subjects, porcine bone is frequently used as a substitute for human bone in forensic cut mark studies (1-3). This appears to have originated as an extrapolation of the use of porcine organs and skin in biomedical studies due to their structural similarities to comparable human tissues. Forensic cut and saw mark studies are increasing in number due to the requirements for validation of methods used in court reporting and availability of new technologies such as three-dimensional microscopy and microCT (4).

Experimental cut mark studies often involve the application of a blade or tool to bone to create indentation or kerf marks. For the purposes of this study we are referring to cut marks as those made by a blade as it passes over the surface of cortical bone without penetrating through to trabecular bone, including kerf marks made by serrated blades or saws where the floor of the kerf can be observed. One of the primary research areas in cut mark analysis is the definition of criteria that can be used to describe and classify them in a standardized manner. The methods used have generally evolved from key principles of the formation of toolmarks outlined by Burd and Kirk in 1942 (5). In the 1990s, Symes pioneered work on the definition of class characteristics for saw marks in bone (6), while Greenfield was developing methods to differentiate stone and metal cut marks on archaeological butchered bones (7). In the 2000s a shift was made from macro- and microscopic examination to exploit new technology such as Scanning Electron and 3D microscopy, while focusing both on the classification and individualization of causative implements. Tennick (8) investigated cut mark features by a wide variety of blade types using light microscopy. However, the author found wide variation in the appearance and depth of cut marks and was not able to identify blades from the kerf features used. Tennick (8) suggested that the use of light microsopy was limiting and that 3D microscopy would be beneficial, and also noted a need to better understand the mechanics of cut mark application. Bello & Soligo (9) devised a method for quantitative analysis of cut marks in cortical bone using Infinite Focus Microscopy, and which was then applied to the study of cut marks made by stone handaxes (10). This method was later adapted by Bonney (11) to discriminate between cut marks made by serrated, non-serrated and bamboo blades for the classification of defleshing marks on trophy skulls. Bailey et al. (12) had some success with eliminating saw blades based on kerf width measured using light microscopy, and Saville et al. (13) were able to match the marks to individual saws using environmental scanning electron microscopy to examine striations within the kerfs.

Despite the established history of forensic cut mark research, biomechanical validation of the porcine model for such studies has not been forthcoming. The mechanical properties of bone define the manner in which it responds to applied forces, and an understanding of these properties is fundamental to the analysis and interpretation of tool marks. Additionally, there are a number of factors affecting inherent mechanical properties of bone that should be taken into account when choosing a subject, including physiological loading and age of the individual. A novel study by Braun (14) investigated the mechanical properties of bovine femora and stone tools, to further understand the relationship between cut marks and the implements that form them but literature to mechanically validate the use of the porcine model is sparse.

Microhardness testing can be used to investigate the mechanical competence of cortical bone. This involves the application of an indenter under constant load to the material being tested, and is a reliable indicator of the degree of mineralization (15). Changes have been observed in hardness of cortical bone along the length of long bone diaphyses and it has been hypothesized that this may be caused by maturation patterns, i.e. the ossification center mineralizes in an outwards direction from the center of the diaphysis, resulting in 'older' bone being present in the central portion, where cortical bone has been measured as hardest (16). Adaptive remodeling has also been suggested as a cause for this observed pattern as it corresponds to the physiological strain distribution in life (6). Microhardness and ash content has also been found to peak at the central diaphyseal region in porcine femora (17).

By studying the variation in mechanical properties in both adult and non-ambulatory stillborn infant porcine long bones, it may be possible to infer whether regional properties are indeed caused by inherent anatomical genetic or loading influences. By comparing these data to that for humans from the literature, it should also be possible to determine whether porcine bone is an acceptable analogue for human bone in experimental cut mark studies.

Materials and Methods

Humeri and femora were obtained from four sows aged over three years and weighing between 185 and 324 kg that showed no evidence of limb deformity or disease, and had been euthanized or died naturally due to problems arising during parturition. Four infant specimens were also used, these were stillborn or died up to 36 hours after birth, weighed between 0.81 kg and 0.95 kg and were collected and frozen at -18 °C within 24 hours of death. Limbs were removed from the adult specimens within 24 hours of death and immediately frozen at -18 °C.

The adult left femora and humeri were removed, manually defleshed and cut into sections using a band saw. The epiphyses were removed and transverse sections, approximately 5 mm thick, were cut at proximal, middle and distal sections of the femoral and humeral shaft. The infant bones were defleshed manually and the epiphyseal regions removed.

The samples from the adults, and the infant diaphyses were degreased by immersing in a mixture of xylene, chloroform and ethanol (1 : 4.5 : 4.5) for 72 hours. This prevents the indentations produced in hardness testing infilling with grease that can make visualization and measurement challenging (18).   Samples were then embedded in Acrulite resin (Rubert and Co. Ltd., Cheadle, UK). Carbon powder was added when mixing the Acrulite resin to provide maximum definition between the bone and resin surfaces (Figs. 1 and 2). In order to provide support during sectioning, the diaphyses from the infant individuals were embedded within a drilled 12 mm cavity in 22 mm dowel (Fig. 1). After the resin had set, the embedded bones were sectioned using a saw microtome (model SP1600, Leica, Germany); with surfaces from the proximal, middle and distal sections of the humerus and femur prepared for testing.

Test surfaces of all samples were polished using successively finer grades of silicon carbide paper (400-, 800- and 1200 grit) and finished with 0.25 µm diamond paste on a dry cotton cloth.

Hardness tests

Samples were tested for Vickers hardness using a diamond tipped pyramidal microindenter (AKASHI M25.4 94898). Indentations were made at least 3 times the width of the indent from the edge of the sample and between 6-8 times from other indents in accordance with ISO 6507-1 (19), to avoid interference between the areas sampled. The adult specimens were tested in the centroids of the anterior, posterior, medial and lateral quadrants. Testing of different quadrants was not possible with the infant specimens due to the narrow cortical region, which only allowed for one indent between the inner and outer edges when leaving the required margin. Five indents were made per individual in each circumferential region in the adults and at each position along the diaphysis in the infants. All specimens were indented with a load of 0.3 kg for 15 seconds and these indents were measured using a x25 objective. Vickers hardness (low force) (HV), (kg mm^{-2}) was calculated using the following equation:

$$HV = 1.854P/d^2$$

Where P is the applied mass (kg) and d is the length (mm) of the indentation diagonal.

Statistical analysis

Analysis of Variance (GLM procedure, *v.*15, Minitab Inc.) was used to investigate regional variation in the cortical bone; inter-animal differences were accounted for in the model by including this as a factor. In order to avoid pseudoreplication (20), analysis was carried out using a single datum (mean of the samples) for each test position with a concomitant reduction in degrees of freedom.

Results

Regional hardness of the Adult femur and humerus

Significant differences were identified in the distribution of hardness within the adult femur. Analysis of the pooled data for each position along the diaphysis showed that cortical bone from the middle region (58.2 ± 1.44 kg mm^{-2}) of the adult femur was harder than the proximal (50.5 ± 0.93 kg mm^{-2}) and distal (48.0 ± 0.29 kg mm^{-2}) regions (Fig. 3, $p < 0.05$; n=4). There were also circumferential differences along the diaphysis; in the middle position, bone from the anterior quadrant was harder than the lateral and posterior and the posterior was less hard than the medial and anterior quadrants (Fig. 3, $p < 0.05$). At the proximal position, bone from the anterior quadrant was harder than that in the other three quadrants and at the distal position the anterior, medial and posterior quadrants were harder than the lateral (Fig. 3, $p < 0.05$).

There was also significant variation along the diaphysis within individual regions; in the anterior quadrant, the middle section was the hardest, followed by the proximal then distal, all were significantly different from each other ($p < 0.05$). In the medial and lateral quadrants, the middle section was harder than the proximal and distal ($p < 0.05$). In the posterior quadrant, there was no significant difference in hardness along the bone (Fig. 3 & 5). When the data for the three positions along the bone was combined, the anterior quadrant was significantly harder than the lateral and posterior quadrants ($p < 0.05$).

There was no significant difference in hardness between the adult humerus (49.6 ± 0.70 kg mm^{-2}) and femur (52.2 ± 1.00 kg mm^{-2}) when pooling the data for region and position ($p > 0.05$, N=4).

In contrast to the femoral data, analysis of the pooled data from the different positions along the humerus showed there was no significant difference between hardness in the middle (49.7± 0.78 kg mm^{-2}) and the distal and proximal regions but that cortical bone in the distal region

(52.2 ±0.83 kg mm^{-2}) was significantly harder than the proximal region (46.9 ± 1.70 kg mm^{-2}) (Fig. 4, p = 0.03, N = 4).

There were also circumferential differences along the diaphysis, but only in the mid and distal diaphysis. In the middle of the diaphysis, the posterior quadrant was harder than the lateral (Fig. 4, $p < 0.05$) and at the distal position, the medial quadrant was harder than the lateral and anterior quadrants (Fig. 4, $p < 0.05$).

Infant humerus and femur

The infant humerus (23.9± 1.37 kg mm^{-2}) was significantly harder than the femur (17.4 ± 0.96 kg mm^{-2}) (Fig. 6, $p < 0.05$). While the middle section of the femur appears to be harder than the proximal and distal sections, there were no significant differences in hardness along the infant femur (Fig. 6, p =0.2, N=4) or humerus (Fig. 6, p = 0.8, N=4).

Age related differences in cortical bone hardness

There was a significant difference in the hardness of adult and infant humeri (49.6 ± 0.70 kg mm^{-2} and 23.9 ± 1.37 kg mm^{-2}, respectively), and adult and infant femora (52.2 ± 1.00 kg mm^{-2} and 17.4 ± 0.96 kg mm^{-2} respectively) when pooling the data for all regions and positions ($p < 0.05$).

Discussion

The hardness of the porcine adult femur (52.23 ± 1.00 kg mm^{-2}) was similar to the values for cortical bone from the femora of sows reported in the literature of 53.5-61.6 kg mm^{-2} (17). The hardness of cortical bone from the adult porcine femur and humerus was comparable to the hardness of adult (aged 35) human fibular cortical bone (55.1 kg mm^{-2}) as determined by Weaver

(15), human iliac and calcaneal bone (49.30 kg mm^{-2}) from older adults free from apparent bone disease (21), and by Wu et al. for human radial diaphyses (43.82 mm^{-2}) (22). Comparable hardness for human (39.4 kg mm^{-2}) and porcine (37.1 kg mm^{-2}) cortical bone has also been reported by Saville et al. (13), although the values were somewhat lower than those reported in the literature for porcine and human bone, and those observed in this study. This may be due to differences in dehydration effects for human samples, or age and sex differences for the porcine samples that were not described.

There were considerable regional differences in the hardness of cortical bone in both the adult porcine fore and hind limb. Hardness varied both along and around the diaphysis in the adult femur and humerus. In the femur the cortical bone was hardest in the middle of the diaphysis with hardness values around 15-21 % higher than the proximal and distal regions, but this was not replicated in the humerus where cortical bone in the distal region was significantly harder than the proximal region (Fig. 5). This mirrors the patterns observed in earlier work on human cortical bone where Evans & Lebow (23) found that the human femur is also hardest in the middle third of the shaft, and the work by Wu et al. who found similar patterns of hardness in the human radius (22). Weaver (15) noted little variation in hardness along the length of the diaphyseal region of the human fibula but found a ‘pronounced’ decrease in hardness in the metaphyseal and epiphyseal region. However, the fibula typically bears very little weight as it functions primarily as an attachment site for muscles, which might explain the relatively uniform hardness along its length.

Significant differences were also found in the hardness of cortical bone around the diaphysis. In the femur, the anterior quadrant was the hardest in the proximal and middle, but not the distal position, whereas in the humerus the posterior quadrant had the highest hardness values (Fig. 5). The anterior quadrant was also the hardest with all three longitudinal positions combined in the femur, whereas the humerus did not show any significant differences in hardness around its circumference with the positions combined. This may be a result of varying in vivo loading patterns.

Differences in gross morphology and shape may also lead to differential loading of the anterior and posterior aspects. The effects of bone curvature and in vivo loading has previously been reported in the equine radius by Currey (25) where the stiffness, strength and histology was shown to vary between the anterior and posterior regions. Previous studies have shown significant differences in the regional variation of other mechanical properties including Young's Modulus and fracture toughness in pigs and humans (26-28) that may have implications for whole-bone or whole-body experiments such as fracturing or blast trauma.

Interestingly, there was no overall difference in hardness between the adult femur and humerus. Previous work on human cortical bone also found hardness values did not vary between long bone types in a single human individual (24). In some ways this challenges the assumption that loading affects adaptive remodeling and mineralization, and hence hardness, and that physiologically loaded limbs from a quadrupedal animal make poor models for unloaded limbs in humans. However, other studies have found hardness of cortical bone to vary widely across different sites within individuals, but not from standardized sites between different individuals (15).

There was however a significant difference between the hardness of the humerus and femur in infant specimens with the humerus having around 37 % higher hardness values compared to the femur. This may be explained by the initiation of ossification of the fetal humerus occurring slightly ahead of that of the femur (29). The use of a quadrupedal animal as a model for human bone is often unaccounted for in studies, however the common use of unloaded bones such as ribs may mitigate for this. However, many commercially available pigs in Europe are slaughtered at a comparable average weight to an adult human (80 kg (30)) but before reaching maturity. The differences observed in patterns of hardness in the fore- and hind-limbs of the porcine specimens might be explained by further investigation of their locomotion and gait, and by studies with finer resolution of age to determine the point at which the difference in hardness of the fore- and hind-limb closes. It has been previously demonstrated that bone hardness correlates with mineralization and age (15,17,31) and that hardness and mineralization reaches a peak at skeletal maturity (around 30 years in humans).

Studies on the mechanical properties of human bone are often skewed toward older age groups due to the natural bias of specimens available for testing – either through death or specimens from living donors undergoing orthopedic surgery. Conversely, older porcine material is rarely available as the animals are not kept beyond their reproductive years, and don't live into senescence. There is limited opportunity for direct comparison and the bias of datasets is a crucial limiting factor for consideration. Likewise, the comparison of data from infant humans and pigs is limited by the precocial versus altricial ambulation (i.e. almost immediately mobile and weight bearing vs. slower development of independent mobility) that is likely to lead to differences in the adaptive responses and therefore mechanical properties. By using bone from individuals of less than 36 hours age at death for the infant porcine specimens in this study, the influences of adaptive changes due to load history are effectively eliminated, as are those of growth rate.

The mechanical behavior of bone as a material should be fundamental to the choice of substitute and methodology in cut mark and trauma studies. Methodological considerations that affect bone mechanics and may require mitigation include preservation method (freezing/thawing cycles, drying/wetting, post-mortem interval) and heating from processing of samples (drilling/sawing, maceration). The age of individual animals used as comparative material should be taken into account, and appropriate parameters chosen according to study methodology and proposed application. This study was limited to infant and adult pigs, and consideration should be given to future work to collect data across a finer resolution of age ranges, particularly to determine how the differences between fore- and hind-limb change with chronological age..

Previous studies on the regional mechanical and physical properties of porcine limb bones including elasticity, toughness, porosity, density and hardness suggest that the variation of these properties within individual bones differ substantially from the patterns observed in humans (23, 26-

28). These variations are likely to affect the way bones behave in some whole-bone trauma studies, such as the identification of fracture characteristics or blast trauma morphology, and porcine bones may be poor substitutes for human in these circumstances. Future work should further investigate the properties of commonly used unloaded bones such as pig skulls and ribs, to determine their suitability as analogues for human bone in forensic cut mark studies.

Acknowledgements

The authors would like to thank Caroline Sims for her assistance with acquiring and processing the pigs, and the reviewers for their thoughtful and constructive comments on the manuscript.

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Figure Legends

FIGURE 1 - Embedded and polished infant porcine humerus specimen.

FIGURE 2 - Embedded and polished adult porcine humerus specimen.

FIGURE 3 - Regional variation in the hardness of cortical bone along and around the adult porcine femur. Vertical bars represent the standard error, n = 4.

FIGURE 4 - Regional variation in the hardness of cortical bone along and around the adult porcine humerus. Vertical bars represent the standard error, n = 4.

FIGURE 5 - Schematic diagram showing hardness along and around the adult porcine humerus (left) and femur (right).

FIGURE 6 - Regional variation in the hardness of cortical bone along the infant porcine humerus and femur. Vertical bars represent the standard error, n = 4.

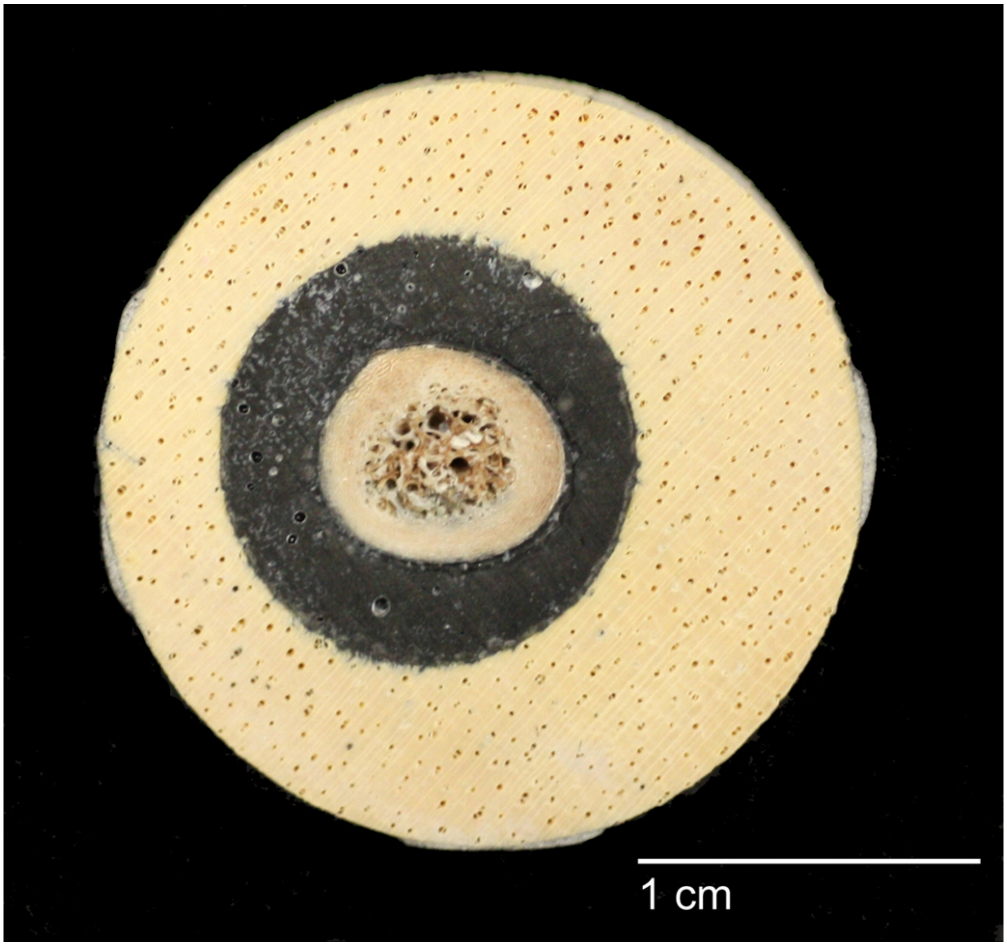

FIGURE 1 - Embedded and polished infant porcine humerus specimen.

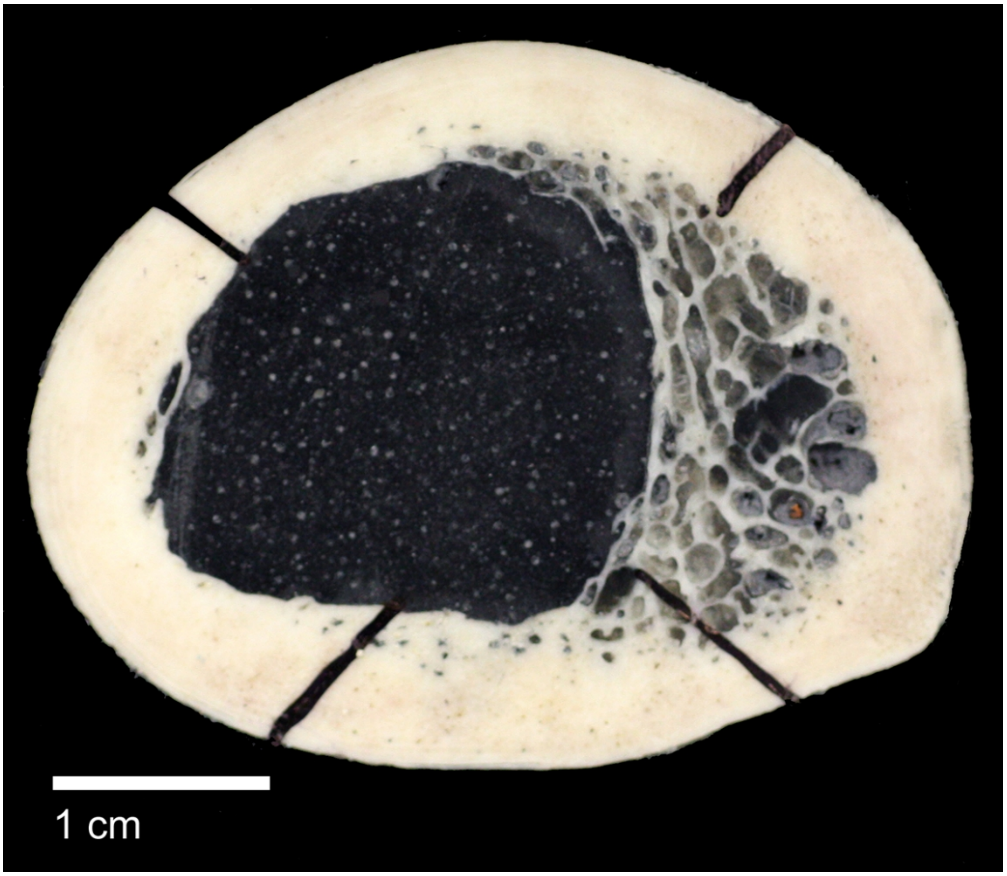

FIGURE 2 - Embedded and polished adult porcine humerus specimen.

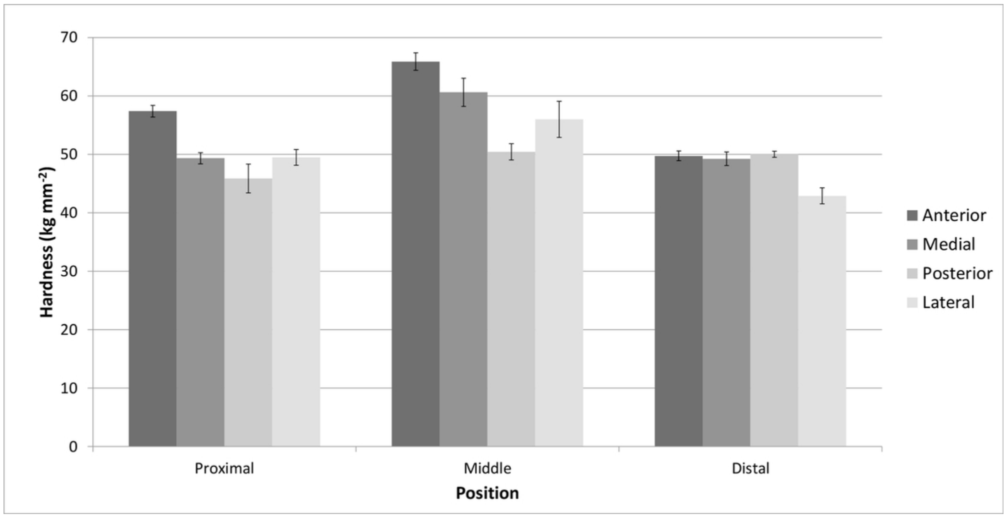

FIGURE 3 - Regional variation in the hardness of cortical bone along and around the adult porcine femur. Vertical bars represent the standard error, n = 4.

99x51mm (300 x 300 DPI)

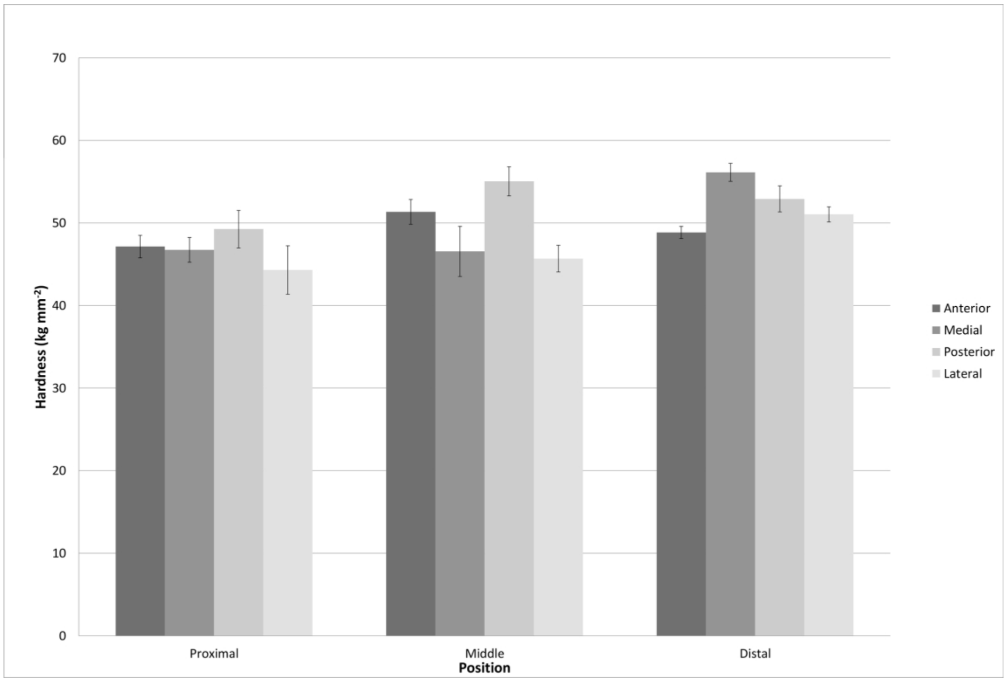

FIGURE 4 - Regional variation in the hardness of cortical bone along and around the adult porcine humerus. Vertical bars represent the standard error, n = 4.

183x123mm (300 x 300 DPI)

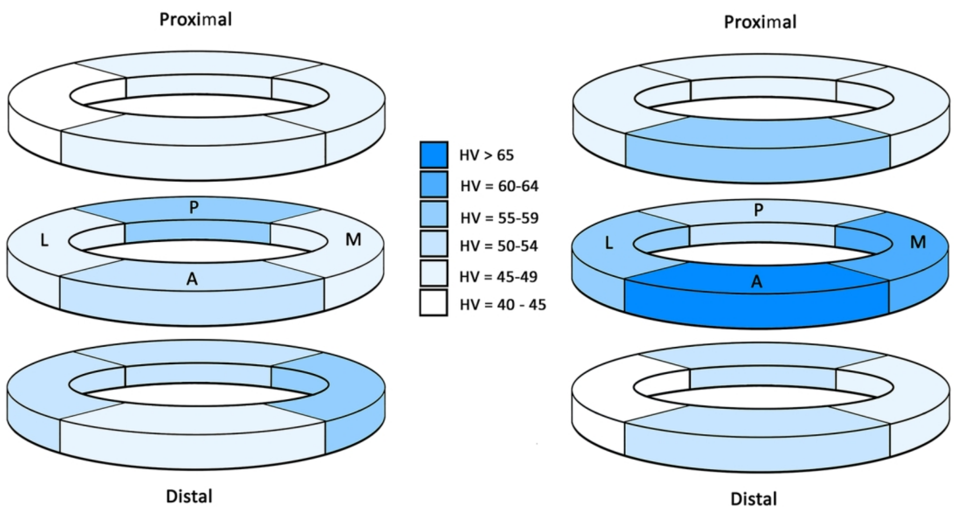

FIGURE 5 - Schematic diagram showing hardness along and around the adult porcine humerus (left) and femur (right).

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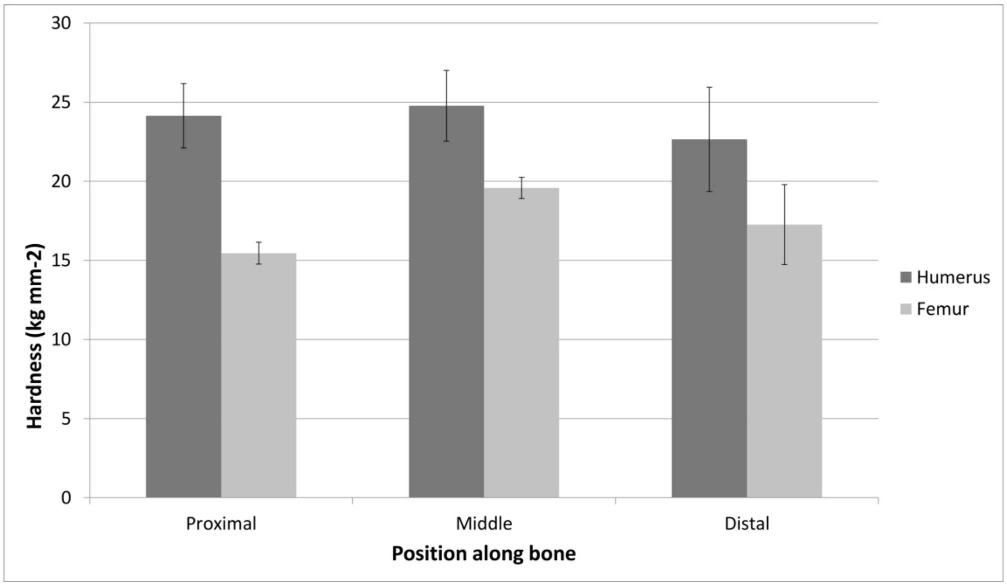

FIGURE 6 - Regional variation in the hardness of cortical bone along the infant porcine humerus and femur. Vertical bars represent the standard error, n = 4.

183x106mm (300 x 300 DPI)